Chapter 16

Laboratory Procedures and Reporting

Analysis begins in the field and extends to the laboratory. The procedures discussed in Chapter 15 are related primarily to the exposure and recovery of skeletal remains. There, we traced remains from their point of discovery into the laboratory. Now we introduce laboratory procedures commonly applied to human osteological material and consider the elements of effective reporting of analytical results. It is important to note, particularly for forensic settings, that Standard Operating Procedures (SOPs) for agencies involved in cases with evidentiary human remains must be followed very closely (see Chapter 15). The chain of custody must always be rigorously respected and documented, and security of the remains vigilantly maintained.

16.1 Setting

Sound procedures, an appropriate setting, and careful use of the proper equipment are essential in osteological analysis, not only to ensure accurate results but also to safeguard the skeletal remains. Of primary importance is that work be conducted over a padded surface. Care should be taken to prevent specimens from contacting hard surfaces or rolling onto the floor. Even well-preserved bones that seem sturdy may be fragile when compared to the instruments used to measure them. The osteologist must therefore be careful not to crush, pierce, scratch, or otherwise damage the specimen with the instruments. Poking or prodding the skeletal material with the fingers can also easily damage the bone, especially the more fragile parts of the cranium.

The study of bones is best done in a well-lit laboratory (Figure 16.1). Lighting is critical. Overhead fluorescent lights are poor for osteological work because they tend to fill the room with diffuse light. Observation of osteological detail depends on the control of incident light on the specimen, and for this reason a swing-arm fixture with an incandescent, halogen, compact fluorescent, or LED light source is recommended for osteological analysis. A unidirectional light source makes it possible for the researcher to highlight subtle bony features or modifications by angling the light to enhance the visibility of surface detail.

It must be noted that there may be several biological and chemical hazards involved with recovery and analysis of skeletal remains, particularly in forensic contexts (Galloway and Snodgrass, 1998). The fungal spores that cause Valley Fever (coccidioidomycosis), commonly found in the soil of southwestern North America and parts of Central and South America, may adhere to archaeological remains (Petersen et al., 2004; Lacy and Swatek, 1974; Werner et al., 1972). Arsenic and organic pesticides may also be present, derived either from soil associated with the remains or from museum pest management practices (Arriaza and Pfister, 2007).

Figure 16.1 **Osteological labwork.** In the laboratory, osteological specimens are fully labeled and restored. Here the osteologist checks for joins. When joins are found, the bones are glued together and temporarily supported in a sandbox while the glue sets. Comparative material should be accessible during these operations. The washing and drying of additional specimens proceeds in the background.

16.2 Stabilization

The strengthening of bones can be accomplished in several ways during and after recovery. Various consolidants (or "hardeners") are available. These are usually either water- or acetone-soluble; they include polyvinyl acetates (PVAs, such as Vinac, soluble in acetone), Paraloid B-72, polymethylmethacrylate (PMM) resins such as Bedacryl 122x (which can be purchased in hard chunks that are then dissolved in an organic solvent), Butvar B-76 (a powder mixed in solvent), and many others. Cyanoacrylate glues of various viscosities are strong, fast-setting, and difficult to remove, but may be necessary to apply before the removal of remains, particularly heavily burned or ashed tooth crowns in forensic contexts (Mincer et al., 1990; Fairgrieve, 2008). Kres and Lovell (1995) and Johnson (2001) review modern consolidants used in osteological work and Rossi et al. (2004) consider these in relation to fragile cremated remains and thin sectioning. Odegaard and Cassman (2007: 85) warn, "a consolidation treatment on inherently fragile material is a decision that cannot be taken lightly since it comes with permanent repercussions. It should not be considered a reversible procedure." Consult with the conservator of the institution that will house the osteological material about the kind of consolidant preferred.

The key to using any consolidant is correct dilution. The most frequent failure in consolidant use is the failure to dilute the solution enough. This results in poor penetration and the formation of a hard outer "skin" on the specimen but a lack of internal hardening. Impregnation with a consolidant having the consistency of water is recommended, usually about a 5–10% solution.

It is usually best to dip whole specimens into the solution and then let them dry on a wire screen. In the case of more fragile remains that will not stand up to immersion, drip the solution onto the specimen. Use organic solvents ("thinners") with extreme caution. Many of these chemicals are dangerous; avoid breathing their fumes and remember that they are often extremely dangerous because of their flammability.

A record of any treatments done to a bone — from the simple (*e.g.,* washing) to the more complex (*e.g.,* consolidation) should be carefully recorded. Note the exact chemical composition of the products used, and on which portion(s) of the remains the treatment was applied. This information should then be filed with the permanent records at the final repository for the remains. This information is vital for future research, as some treatments may detrimentally affect the ability to use methods such as ^{14}C dating (D'Elia et al., 2007) and DNA analysis (Nicholson et al., 2002; Vuissoz and Gilbert, 2007).

16.3 Preparation

Techniques used in skeletal preparation vary according to the condition of the bone and the context of its discovery. Archaeological bones, bones from forensic cases, and fossilized bones are all prepared differently. The preparation of any of these types of remains has its own requirements and is subject to specific restrictions. Preparation of all of these types of remains will be considered below.

16.3.1 Preparing Archaeological Bones

Washing bones in water dissolves and degrades DNA (Pruvost et al., 2007), may initiate chemical reactions within the bone, and is generally an irreversible, invasive action. It should only be done sparingly, after careful consideration, and with proper documentation (Odegaard and Cassman, 2007).

If you determine that the bone must be washed, be sure it is well-preserved and verify that no adhering substances valuable for later analysis, such as textile, ochre, pollen, or metal residues, are present. Wash bones in lukewarm water (without detergents or any other additives) using soft brushes, wooden probes, and spray bottles. Never wash more than one skeleton at a time. Use a screen in all washing, field and laboratory, to keep small bones from being lost. As the washing water becomes muddy, small fragments may detach and become lost in the sediment at the bottom of the basin or disappear down the drain. Clean the basin and screen frequently, making sure that both are checked between processing each burial. Depending on humidity, the washed bones dry in 24 to 48 hours on wire racks in the shade. In the laboratory, you can speed this up by using a fan to gently blow air across racks of drying bones. Never use a heat source due to the danger of bone surface exfoliation.

Whereas the washing of bones should be minimized, there are many good reasons to remove adhering soil from archaeological bones. Soil and other adhering matrix obscures morphology and can make analysis difficult or impossible. Large clumps of soil, if left attached to a bone, can unexpectedly detach, exfoliating the surface of the bone. They can also scratch or damage other bones when moved during storage and transport.

Bone can be cleaned without water using soft-bristle brushes. Before cleaning, set out a large sheet of paper to catch removed material. After cleaning, roll the paper into a funnel shape and decant the shed material into an appropriate labeled container (if the material is dry, a zip-lock plastic bag is commonly used) to be kept with the bone(s), preserving it for potential future research. Large clumps of hardened soil may need to be lightly moistened in order to be gently removed from the bone.

16.3.2 Preparing Forensic Bones

Forensic bones will sometimes have adhering flesh and other tissues that make direct observation of the bone difficult, if they are not removed. The process of removing soft tissue remnants from bone is called **maceration**. Before undertaking maceration of forensic remains, there are three important factors to consider: future DNA testing, the possible evidentiary value of the tissue to be removed, and the chain of custody.

To preserve the possibility of downstream DNA testing, traditional defleshing techniques such as those used for building comparative mammalian skeletal collections (*e.g.,* Hildebrand, 1968; Mori, 1970) are not recommended. Rennick et al. (2005) note that the use of bleach or prolonged exposure to boiling water has a degradative effect on bone DNA, but found that the use of milder agents such as powdered detergent or sodium carbonate was less damaging. Fenton et al. (2003) and Steadman et al. (2006) found that shorter duration exposure to slightly lower temperature (90° C) water was less destructive to bone DNA than many traditionally "conservative" maceration techniques.

Tissues of any kind should be regarded as potential evidence in forensic cases. Be sure to consult a soft tissue pathologist before removing soft tissues. When possible, select a maceration technique that will allow the removed tissue to be preserved. In many cases this will mean physically macerating the bulk of the tissues by removing them carefully with appropriate dissecting tools. Never destroy evidence of any kind; even insect larvae contained in these tissues may provide important clues in a forensic case.

Finally, the chain of custody must be maintained with all physical evidence in a forensic case. Melbye and Jimenez (1997) discuss the implications of chain of custody in forensic osteology. As stated by Komar and Buikstra (2008: 99), maintaining the chain of custody requires that:

- the investigator is aware of the exact location of the evidence at all times;
- the evidence is maintained in a secure location;
- the evidence is sealed to prevent tampering;
- access to the evidence is restricted to the responsible investigator or authorized designates;
- any handling, transport, analysis, or examination of the evidence is acknowledged in a written log.

16.3.3 Preparing Fossilized Bones

For fossils, more specialized preparation techniques are often called for. Fossils are sometimes encased in a very hard **matrix** (surrounding material) that may be even harder than the bone itself. Sometimes the matrix can be softened with solvents such as acetone, paint thinner, or even water. Very important fossil specimens should be molded (see Section 16.10) before cleaning to make a record of pre-preparation status. When cleaning, matrix samples should be kept so that future investigators might be able to establish **provenience**, the stratigraphic and spatial position of the specimen.

Some commonly used fossil preparation tools and techniques include the following:

- **Hammer and chisel.** This technique has a long history, and many fossils have the scars to prove it. Speed is an asset, but the shock imposed on the specimen and the lack of fine control are negative points.
- **Dental drill.** This fast but dangerous technique has been used for many years. Positive points include good cutting power, more control than a hammer and chisel, and lack of shock to the specimen. However, extreme care must be taken to keep the surface of the grinder from drilling into the surface of the object being cleaned.

Figure 16.2 **Preparation-related damage on the Pleistocene fossil *Homo* cranium from Petralona, Greece.** Removal of matrix by a high-speed grinding wheel has produced damage on the original surfaces of the left nasal bone, and chiseling marks are seen above the left orbit. Natural size.

- **Dental pick (or needle held in vise) under binocular microscope.** This is often the most effective way to clean a fossil; it gives the preparator much control and limits potential damage to tiny areas. However, this work requires an enormous amount of time and patience.
- **Acid treatment.** Dilute acetic, formic, or hydrochloric acids can be used to dissolve matrix holding some fossils. Excellent detail may be obtained by this technique, but it calls for patient, extended monitoring to keep the acid from attacking the specimen itself, etching its surface, or weakening its structural integrity. Take all standard laboratory precautions when using acids. Use this method only after consulting an experienced preparator or conservator.
- **Air abrasion.** This preparation technique uses a tool that shoots a stream of particles at the matrix like a miniature sandblaster. This provides speed and control without shock. However, the abrasive particles may obscure detail and "frost" the bone and tooth surfaces. In addition, it is difficult to control the abrasive stream as it ramifies through cracks below the surface of a specimen.

The most essential ingredients in successful fossil preparation are patience and experience, but help and advice from skilled preparators are crucial. Note that all of the techniques discussed here carry with them hazards for the bones or fossils. It is better to be safe than sorry in preparing important specimens. Preparation trauma that is readily observable on previously cleaned bones and fossils shows that many past workers were not careful enough (Figures 16.2–16.3).

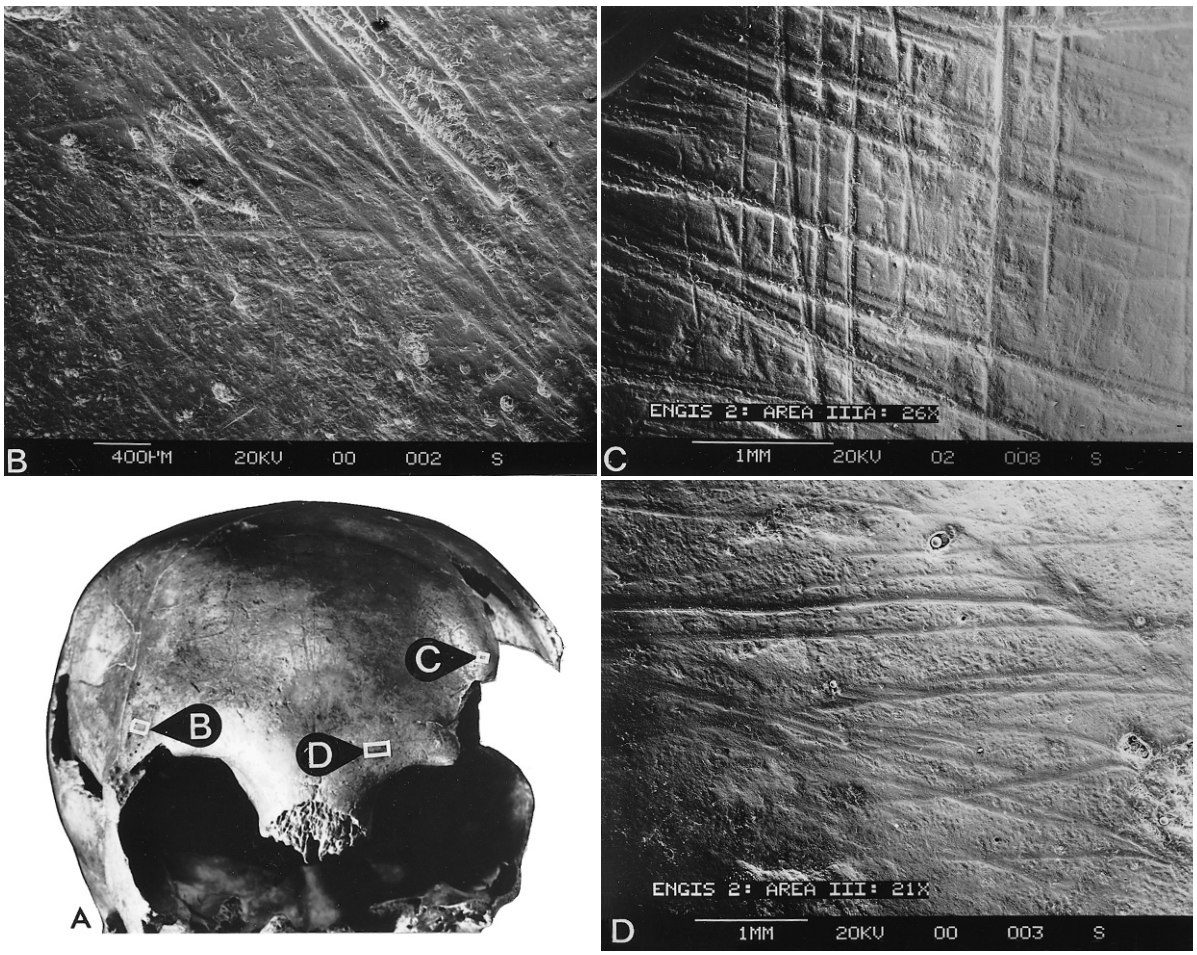

Figure 16.3 **Taphonomic or preparation damage?** Frontal view of an immature Neanderthal cranium from Engis, Belgium (A). The superficial scratches along the midline were made by a diagraph instrument needle, and those along the broken edge of the left frontal were made by sandpaper used to smooth the previously plaster-reconstructed area behind. These marks were interpreted as evidence of Neanderthal mortuary practice (Russell and LeMort, 1986). This interpretation was shown to be mistaken by White and Toth (1989). Scanning electron micrographs (B–D) show the sandpaper striations. Note the "doubling back" at the end of each sandpaper grain's path as the end of the sanding stroke was reached. These figures illustrate the utility of the scanning electron microscope in investigating bone modification at magnification.

16.4 Restoration

Restoration involves putting pieces of broken bones back together. A detailed knowledge of osteology greatly simplifies this process; the ability to identify the side and position of fragments allows the quick identification of joins. The restoration of fragmentary bones is often described as more difficult than doing a jigsaw puzzle. This is an exaggeration. A broken skeleton has one more dimension and far more information than a picture puzzle of a polar bear in a snowstorm. Restoration is often quick and easy for the competent osteologist (see Figure 16.1 for an illustration of restoration in progress). The following are valuable guidelines for restoration:

- Use a glue that may be dissolved later. This ensures future workers the ability to correct any unintentional mistakes of restoration.
- Do not hurry. As in preparation, patience and experience are essential in good skeletal restoration.

- Restore the face and vault separately before joining them. Use the mandibular condyles as a guide to restoring the correct cranial breadth when this is in doubt.
- Be sure the bones to be glued together are dry, unless a water-soluble glue is being used.
- Do not glue until you are positive of a good join. Check under the microscope if necessary.
- Make sure the joining surfaces are clean of debris. Adhering grit, consolidant, and flakes of bone can result in misalignment of broken pieces.
- Use color, texture, and — most importantly — anatomy to match the pieces.
- Never glue teeth into their sockets until you are absolutely positive that they belong there. Interproximal contact facets provide an invaluable guide to accurate tooth placement.
- Do not glue yourself into a corner by leaving unfilled gaps between bones. Instead, use removable painter's masking tape to make temporary joins. Do not leave the masking tape on for more than a couple of months and be careful that the bone surface can release the tape without exfoliating. When satisfied that the joins are correct, progressively remove the tape and glue the broken surfaces together.
- Reconstruct only where necessary. Use soft plaster or a 50:50 mixture of paraffin wax and dry plaster heated to a liquid in a saucepan on a hotplate (not an open flame). Do not ignite the paraffin. This restoration material is easy to work with and remove. In contrast, modeling clays (plasticine) tend to be more greasy and should generally not be used, except as temporary props. After the restoration is complete, be sure to demarcate the reconstructed from the real surfaces. Reconstruction (as opposed to restoration) is rarely justifiable for an original specimen because it is subjective and it obscures valuable cross-sectional information.
- Use a sandbox and gravity to position pieces while rebuilding them. Anchor one piece in the sand and balance the other piece on top of it, perhaps temporarily supporting the glue join with removable painter's masking tape (Figure 16.1). Be sure to let the glue completely harden before removing the piece from the sandbox.
- Where contacts are limited and weak, brace the parts by using struts made of wooden or glass rods.
- Do not use glues, consolidants, or reconstruction material that will inhibit molding rubbers that may be used on the specimen at a later time. Check any such substances for compatibility with molding rubber before applying them to the specimen.
- For some specimens, complete restoration is extremely difficult; some distortion is the result of warping rather than fracture. Such warping is impossible to correct.

16.5 Sorting

The osteologist is often faced with the challenge of sorting a collection of bones that contains more than one skeletal individual. Of primary importance in this sorting are age, size, and sex differences as well as bilateral, nonmetric traits. Matching articular or interproximal facets often provides clues about association. Preservational factors such as bone color, weathering, or integrity are of secondary importance, but are sometimes useful in sorting individuals.

The **minimum number of individuals** (**MNI**) in any assemblage of bones is the minimum number of individuals necessary to account for all of the elements in the assemblage. A very simple example might be an assemblage consisting of two specimens, a fragment of left humeral head and a fragment of left distal humerus. These two specimens, even though the intervening shaft is missing, *could* represent the same individual (unless they are of patently different individual ages). Even though there might be two individuals involved, the MNI value would, in this case, be one.

This basic logic is used to determine the MNI value for any assemblage of human remains by following these procedures:

- Remove all nonhuman elements.
- Separate the bones according to element and side.
- Within each right-side element category, count the minimum number of individuals—not pieces—represented. Consider all possible joins between fragments and assess the age-at-death of each fragment.
- Perform the same minimum number count for left-side bones within each element category and then check for individuals represented by left-side bones that either do match, or possibly belong, to those from the right. These do not increase the count.
- Left-side bones that do not match corresponding right-side ones in age or morphology are added to the minimum number count.

After this is done for all paired and unpaired elements, the greatest minimum number of individuals has been determined. Consider, for example, an assemblage consisting of two right maxillae with full adult dentitions, three left femora of adults, one right femur of an infant, two sacra, four adult right calcanei, and three permanent right upper central incisors. The MNI in this assemblage is six—the infant plus five adults (determined by two right central incisors in maxillae, and three isolated upper right central incisors). The maximum number of individuals is determined by counting all nonjoining, nonmatching elements—in this case, a total of 15. For another example, see Figure 16.4. Note that the MNI is not equal to the "most likely number of individuals" (MLNI), a statistical construct proposed by Adams and Konigsberg (2004, 2008). Outram et al. (2005) discuss the benefits of parallel and integrated analyses of both human and nonhuman bones, especially from sites with highly fragmented remains.

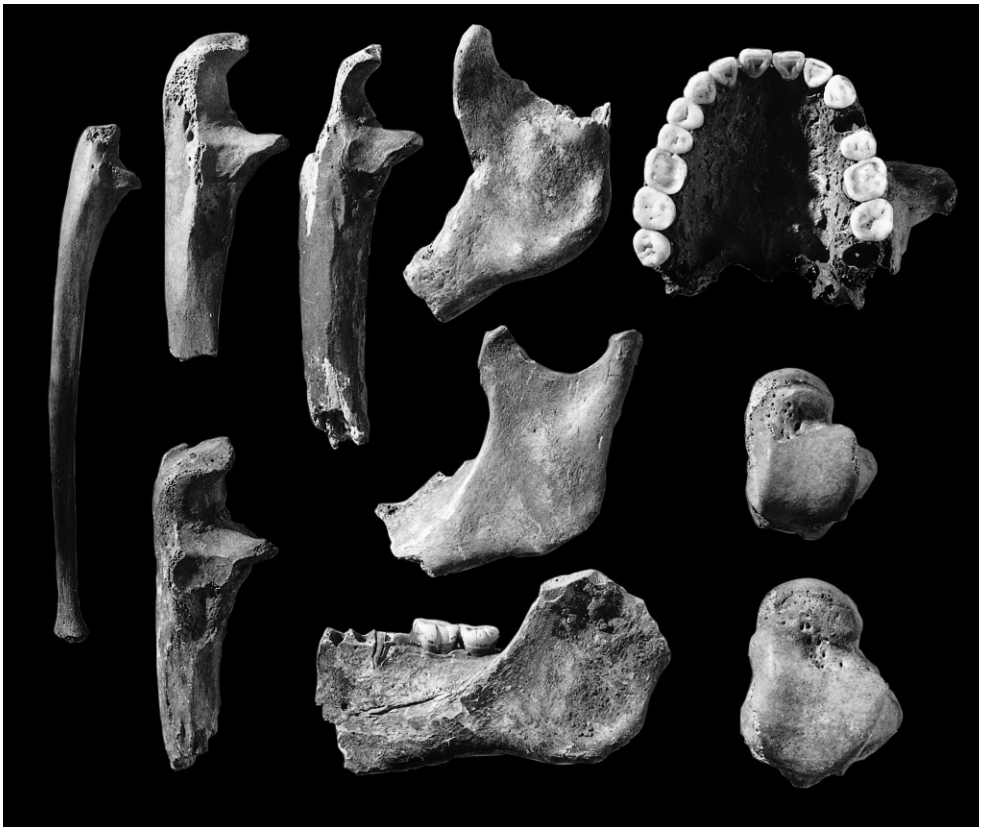

Figure 16.4 **Minimum Number of Individuals (MNI).** To determine the minimum number of individuals for this sample of ten specimens (shown one-half natural size), first note that there are no nonhuman pieces. Sort the pieces by skeletal element and side. There are two right tali, three left mandibles, one maxilla, and four right ulnae. One of the ulnae is immature. The MNI is thus equal to at least four people. Because no pieces join or are antimeres (opposite sides of the same individual), it is possible that each piece represents a different individual, so the maximum number of individuals indicated for this sample is ten.

16.6 Metric Acquisition and Analysis

Because osteological work is part of the scientific enterprise, it is necessary to communicate results to other researchers in an unambiguous and precise manner. One of the most convenient and effective ways to communicate osteological observations is to quantify them—to express them as numbers. Thus, to inform colleagues and others about a particular tooth, it is a simple matter to measure and count characteristics of that tooth.

16.6.1 Traditional Osteometric Tools (Figure 16.5)

Many measuring tools have been invented and developed for osteological analysis. Figure 16.5 illustrates some of the most common of these. Devices for orienting crania are also available, and instruments called diagraphs are used to trace certain profiles of crania held in these devices. Most of these precision instruments are made of steel and are expensive. They are also sharp. Care should be exercised to see that the instruments are not damaged during use. More importantly, because bone is softer than steel, these instruments can scratch or perforate bone surfaces, and care should be taken to see that such damage does not occur during analysis.

a. **Sliding calipers**: The sliding caliper is the most frequently used measuring tool in the osteologist's toolkit. It has a pair of jaws whose variable gape is measured via a dial, a scale, or a digital readout on the caliper shaft.
    1. **Vernier caliper** (Figure 16.5f): Vernier calipers use a combination of a large primary (or fixed) linear scale and a smaller nonlinear (or vernier) sliding scale to measure distances to the nearest 0.1 mm (*i.e.,* they have a measurement error of 0.05 mm). Whole millimeters are read from the primary scale, and fractional millimeters are read from the relative position of the linear and nonlinear scales. As they have no gears, pinions, or dials that may wear or otherwise slip out of alignment, they are very reliable. On the other hand, a certain amount of skill and practice is required, as they take some getting used to. Care must be taken to avoid possible parallax errors on most models.
    2. **Dial caliper** (Figure 16.5g): Dial calipers are a refinement of vernier calipers, replacing the secondary nonlinear sliding scale with a graduated dial. Dial calipers can be used to measure distances to the nearest 0.02 mm (*i.e.,* they have a measurement error of 0.01 mm). Whole millimeters are still read from the primary scale, but fractional millimeters are read from a large dial. Regular alignment ("zeroing") of the dial is a necessary but simple task. Most models of dial caliper are also susceptible to parallax errors (due to the differing heights of the dial arm and the graduated marks on the dial), but these can be overcome with practice and consistent dial-reading technique.
    3. **Digital caliper** (Figure 16.5a, b): Digital calipers are a further refinement of dial (and vernier) calipers, replacing the dial with an electronic digital display from which the entire measurement can be read. Digital calipers can be used to measure distances to the nearest 0.01 mm (*i.e.,* they have a measurement error of 0.005 mm) and, as with dial calipers, regular alignment ("zeroing") of the dial is necessary and simple. Unlike vernier and dial calipers, there is no risk of parallax errors when reading measurements. One particularly useful feature of some digital calipers is the ability to directly interface the caliper with a computer using a built-in RS-232 data port and cable.
    4. **Dental caliper** (Figure 16.5b, f): Dental calipers are specialized versions of the more typical sliding calipers. The primary difference is the replacement of larger, sturdier jaws with either narrower, pointed jaws (Figure 16.5f) or needle points (Figure 16.5b) capable of fitting into small interproximal gaps.
b. **Spreading caliper** (Figure 16.5e): A spreading caliper is usually used for work on cranial anatomy. Spreading calipers consist of two recurved, hinged jaws, often with an integrated

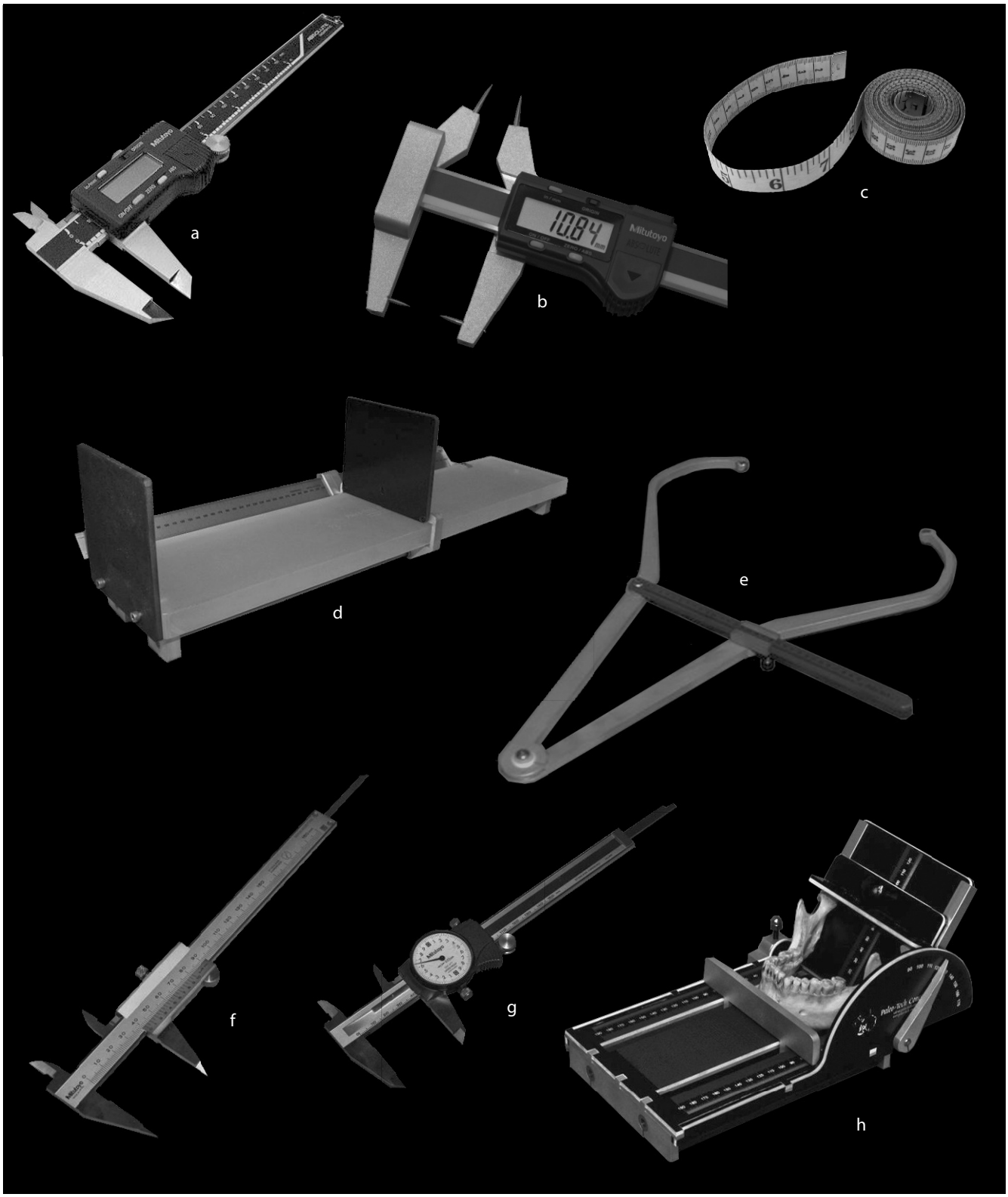

Figure 16.5 **Tools for osteometric measurement.** *Key:* a) digital sliding caliper with standard points; b) digital sliding needle-point caliper; c) cloth measuring tape; d) osteometric board; e) spreading caliper; f) vernier dental caliper; g) dial caliper with standard points; h) mandibulometer.

graduated scale from which measurements can be directly read. Spreading calipers are used to measure distances on large, irregular bones (*e.g.,* cranium and articulated pelvis) that are difficult or impossible to measure with sliding calipers or osteometric boards.

c. **Osteometric board** (or **bone board**) (Figure 16.5d): The osteometric board is second only to sliding calipers in its utility, versatility, and applicability. Osteometric boards are useful in measuring lengths and angles of postcranial elements. An osteometric board is composed of a long, sturdy metal or wooden horizontal plate with an attached perpendicular endplate. A graduated scale extends from the fixed endplate, and there is a second, movable endplate whose position can be read from the graduated scale.

d. **Cloth measuring tape** (Figure 16.5c): A simple cloth tape, graduated in millimeters, is the standard tool used to measure the circumferences of long bones. Care must be taken to find a tape that does not stretch, and some practice is required to get reliable measurements.

e. **Mandibular goniometer**: The mandibular goniometer is a simple device for measuring the angle between two planes. It typically consists of two pieces of wood (or plates of metal), hinged together and connected with a protractor. A mandible is set on the fixed horizontal plate (to which the protractor is fixed), and the movable plate is adjusted so that it contacts the posteriormost points of the condyle and ramus. The mandibular angle is read as the angle between the two plates.

f. **Mandibulometer** (Figure 16.5h): The mandibulometer is an extension of the mandibular goniometer. In addition to mandibular angle, the mandibulometer also measures mandibular length and maximum ramal height.

16.6.2 Analysis

Recent advances in computerized tomographic (CT) and laser scanning technologies have made it possible for osteologists to acquire metrics digitally, and to display them via a computer screen remote to the specimen(s) under study. At first glance, this technological capability brings with it the prospect of fast, easy, and accurate data acquisition and distribution. Rare or valuable specimens can now be digitized by laser, CT scans, and 3-D digitizers (Mafart et al., 2004; Mafart and Delingette, 2002; Ousley and McKoewn, 2001; Zollikofer et al., 1998). So alluring are the siren songs of these new capabilities that some have even suggested that they obviate the need to curate original specimens (see also Chapter 17). This is a mistaken and extremely dangerous philosophy in osteology.

History provides a relevant lesson here. When it became possible to "capture" morphology of fossil specimens via flexible rubber molds and accurate plastic casts, replicas of the fossil originals were distributed throughout the world. These casts are immensely valuable as teaching and preliminary research tools, but experience has shown that they are no substitute for the originals. Distortion, matrix cover, and internal morphology are all lost in a cast. Workers unfamiliar with originals can be badly misled by such features, features only visible with reference to the original. Misinterpretations based on inaccurate observations and measurements on photographs and casts are an embarrassing part of the published literature of human paleontology. See Clarke and Howell (1972) for an analysis of problems inherent in observations and measurements from photographs and casts.

With either two- or three-dimensional digital images scanned from original osteological specimens, these problems are compounded dramatically as the investigator moves another step away from the original. Color, texture, internal anatomy, matrix cover, consolidant cover, preparation damage, erosions, and distortions of all kinds may be faithfully recorded by such imaging of the original, but for the osteologist looking at a computer monitor on the other side of the planet, these features may not be digitally distinguishable from actual bony anatomy. Humans routinely make mistakes, and relying on these technologies risks increasing the number and impact of these mistakes in human osteology. For obvious reasons, osteologists should always refer to the original specimen, even when acquiring metric data from digital sources.

Figure 16.6 **A three-dimensional laser-scanning unit at the University of California, Berkeley.** The machine accurately measures three-dimensional surface topography and sends these data to a computer where measurements, restorations, and other manipulations are possible.

The popular image of an osteologist at work is one of a person in a white laboratory coat, measuring instrument in hand, manipulating some bone (usually a cranium). The role of the professional osteologist is far broader than this. In the formative days of osteology, quantification of bony anatomy was the focus of most work. Elaborate sets of measuring points were defined, and vast quantities of metric data were compiled. Just because a certain measurement has been reported, however, does not mean that it is either useful or even reproducible (Stirland, 1994). Conversely, new metrics are routinely developed to quantify morphological observations. The traditional days in which measurement was done for measurement's sake are thankfully over now, but metric analysis does continue to play a primary role in osteology. Howells (1969a) provides an interesting background for the selection of skeletal metric points.

All scientific measurements, including those in osteology, should be taken in the metric system. This system expresses linear osteological measurements in millimeters, centimeters, and meters. Some observations are possible to quantify even though they are difficult or inappropriate to record as actual measurements. For example, traits such as the Carabelli's cusp on upper molars may be recorded as absent or present. Variables recorded in this way are called **discontinuous** (or **discrete**) **variables**, as opposed to **continuous variables** such as linear measurements.

Metric analysis in osteology requires more than simply measuring a given element. It is critically important to provide precise definitions of each measurement. Furthermore, the degree to which measurements can be reproduced is important in metric analysis. Tables 16.1 and 16.2 demonstrate the proper way to calculate and report measurements and their associated errors. Lyman and VanPool (2009), Buikstra and Ubelaker (1994), and Heathcote (1981) provide further details on measurement error in osteology.

Metric data in osteology are usually compiled as a result of measuring arcs, chords, or volumes. Indices are made by combining these values. For example, the Cranial Index is the product of the maximum cranial breadth (*bi-euryon*) and 100, divided by the maximum cranial length (*glabella* to *opisthocranion*). Indices are convenient because they express shape as a single variable. For

Table 16.1
Measurements: Estimates of Real Dimensions

It is important to understand the difference between the actual dimension you wish to determine and any measurement you take of that dimension. While it may seem straightforward to take a caliper, measure the distance between the two orbits as 41.0 mm, and then state that the interorbital distance is 41 mm, such a methodology is flawed and may result in inaccurate estimations of the actual dimension and/or improper levels of precision in those estimates. Such an approach ignores several factors that must be taken into account:

- The exact definition of the standardized metric—measurements must be taken exactly as defined to be useful in any comparative or descriptive context.
- The precision of the instrument used to take the measurement—generally defined as one-half of the smallest increment the instrument is capable of measuring.
- The effect of random error, an unavoidable factor which is present in all measurements.
- The effect of any systematic error(s), such as improperly zeroed or calibrated calipers, or individual differences in the way a measurement is taken.
- The conventions for presenting the best estimate of the actual metric and the estimated error in that estimate.

You should present your estimates of dimensions in the following form:

$$X = x_{best} \pm \sigma_M$$

where X is the metric being measured, x_{best} is the best estimate of that metric (in practice, a simple average of all measurements taken of that metric), and σ_M is the estimate of the error present in x_{best}. To obtain σ_M, square the differences (**deviations**) of each of the individual measurements from x_{best} (the average of all the individual measurements), and add all of these squared deviations together (this is often referred to as the **sum of squares**). Divide this sum by the number of individual measurements minus 1, and then take the square root of the result. The result is known as the **standard deviation**, or σ. Divide the standard deviation by the square root of the number of individual measurements taken to get σ_M, referred to as the standard error of the mean or, more simply, the **standard error**. See Table 16.2 for an example of how to calculate the standard error.

To convey the correct precision of your estimate, make sure the number of significant digits in your estimate does not exceed the smallest number of significant digits of any of the component measurements. For instance, if you had the following five measurements of the dimension 'Y':

23.25 mm 23.5 mm 23.34 mm 22.97 mm 23.30 mm

your raw calculations for mean and standard error would yield 23.272 and 0.086336551, respectively. The first figure has five significant digits, and the second figure has eight, but your original measurements had either three or four significant digits. As a result, the final statement of your best estimate of the dimension's true size should have only three significant digits:

$$Y = 23.3 \pm 0.0863 \text{ mm}$$

A note for users of digital calipers: As the example above demonstrates, you must exercise caution when recording measurements from your caliper's digital display. If the measurement is given as 50.00 mm, do not abbreviate this to 50.0 mm or 50 mm, as these latter figures do not contain or convey the precision actually obtained in your measurement.

A note for users of nondigital calipers: Users of nondigital calipers should also exercise caution when recording measurements from their calipers. If the dial or scale points to 50.0 mm, do not abbreviate this to 50 mm, as this figure does not contain or convey the precision actually obtained in your measurement. Likewise, do not express a measurement of 50.0 mm as 50.00 mm unless 1) the caliper is intended for this level of precision, and 2) you are confident that you can distinguish 50.00 mm from both 49.99 mm and 50.01 mm.

Table 16.2
Worked Example: Calculating an Estimate and its Standard Error

A. An osteologist measures the buccolingual diameter of a tooth and gets a reading of 10.9 mm. . . . . **Measurement A = 10.9 mm**

B. Time is allowed to pass (best if a few days) so that knowledge of the original measurement does not influence the remeasurement.

C. The same investigator then remeasures the same tooth at 11.0 mm. . . . . **Measurement B = 11.0 mm**

D. Time is again allowed to pass (best if a few days).

E. The same investigator measures the tooth at 11.4 mm. . . . . **Measurement C = 11.4 mm**

F. There are now three measurements. For the best estimate of the actual dimension of the tooth (x_{best}), calculate the mean (average) of these:
- add the three measurements . . . . . **A + B + C = 33.3 mm**
- divide this total by the number of measurements taken (n) . . . . . **33.3 ÷ 3 = 11.1 mm**

G. To assess the degree of error associated with your estimate of the metric, calculate the standard error of the mean:

calculate the deviation of each measurement from the mean
- deviation of the first measurement from the mean . . . . . **11.1 mm − 10.9 mm = 0.2 mm**
- deviation of the second measurement from the mean . . . . . **11.1 mm − 11.0 mm = 0.1 mm**
- deviation of the third measurement from the mean . . . . . **11.1 mm − 11.4 mm = − 0.3 mm**

square each deviation
- square of the first deviation . . . . . **$(0.2\ mm)^2 = 0.04\ mm^2$**
- square of the second deviation . . . . . **$(0.1\ mm)^2 = 0.01\ mm^2$**
- square of the third deviation . . . . . **$(-0.3\ mm)^2 = 0.09\ mm^2$**

• calculate the sum of these squared deviations (the "sum of squares") . . . . . **$0.14\ mm^2$**

calculate the standard deviation (σ)
- divide the sum of squares by $(n-1)$ . . . . . **$0.14\ mm^2 \div 2 = 0.07\ mm^2$**
- σ is the square root of this quotient . . . . . **$\sqrt{0.07\ mm^2} = 0.264575\ mm$**

• the standard error of the mean (σ_M) is σ divided by $\sqrt{n}$ . . . . . **$0.264575\ mm \div \sqrt{3} = 0.152752\ mm$**

• report σ_M rounded to the proper number of significant digits . . . . . **0.153 mm**

These results are then presented in the following form:

Buccolingual diameter = 11.1 ± 0.153 mm

By calculating and reporting the values in this way, the osteologist conveys a clear and unambiguous statistical message about what the actual value of the dimension is likely to be, how accurate (or repeatable) the measurements were, and what degree of precision was used in taking the measurements. Because the results are presented in an accepted, standardized, statistically meaningful way, the values can be plugged into other standard statistical equations. For instance, if we want to know with 95% certainty what the actual value is (assuming that the measurements taken have a normal distribution), we can easily calculate the 95% confidence intervals using the equations:

$$\text{upper 95\% limit} = x_{best} + (\sigma_M \times 1.96)$$
$$\text{lower 95\% limit} = x_{best} - (\sigma_M \times 1.96)$$

Thus, we are able to say with 95% certainty that the actual value of the metric lies between 10.8 and 11.4 mm.

example, a short or "broad" skull has a higher index than a long, narrow skull. A selection of useful measurements and indices are listed in the "Measurements" sections in Chapters 4–13. In addition, Bass (2005) provides a concise guide to standard measurements and indices most widely used in human osteology, and Buikstra and Ubelaker (1994) recommend a set of 34 cranial and 44 postcranial measurements to be taken on intact skeletons.

Expression of skeletal element shape, or morphology, can be examined by univariate (single measurement), bivariate (two measurements), or multivariate (three or more measurements) statistics. The successes and failures of multivariate analyses in human osteology and paleontology are examined by Lovejoy (1978), Howells (1973), Frayer (1985), Reyment et al. (1984), and Corruccini (1978, 1987). The use of measurements such as linear distances, angles, and indices is commonly referred to as traditional morphometrics. Geometric, or modern, morphometrics is a collection of methods for acquiring and analyzing data that more fully retain geometric information about the shapes or structures under study; *e.g.,* the Cartesian coordinates of landmark point locations. These data are most easily obtained via 3-D digitizers or scanners, or in two dimensions from dimensionally accurate digital photos. Bookstein (1991) and Rohlf and Bookstein (1990) provide introductions to the techniques of traditional morphometrics, and Dryden and Mardia (1998) offer an in-depth examination of the mathematical and statistical foundations of geometric morphometrics. Richtsmeier et al. (1992, 2002) provide a review of anthropological morphometrics. Lawing and Polly (2010) offer a good introduction to the modern application of geometric morphometrics to broader questions. Robb (2000) provides a good introduction to the tools that human osteologists will need to do more than just *acquire* data. The thoughtful and appropriate *analysis* of those data is not a trivial endeavor.

16.7 Photography

Osteologists use photography to provide an **archive** (record) of bony material and its context as well as to communicate information in publications and presentations. Maximizing the utility of photography in osteology assumes an understanding of the medium that goes beyond the abilities of the casual photographer.

The advent of digital photography has changed the face of scientific imaging and has all but supplanted the use of film. Digital imaging brings with it many benefits as well as a number of important considerations. Understanding the technology and the differences between digital and film photography is important. However, an adequate discussion of this is beyond the scope of this book. Trussel and Vrhel (2008) and Sedgewick (2008) provide comprehensive overviews of the fundamentals of digital imaging and digital image processing. Offered here are a few general considerations regarding photography in osteology.

16.7.1 Equipment

Several kinds of still-image cameras exist (*e.g.,* point and shoot, single-lens reflex, rangefinder), but a single-lens reflex camera (SLR, or its digital equivalent, DSLR) will satisfy most situations in osteology. Unlike point-and-shoot cameras, SLR cameras feature interchangeable lenses, minimal shutter lag, and give the photographer full control over aperture, shutter speed, and focus.

DSLR cameras that use "full-frame" digital image sensors (similar to the 24× 36 mm active area of 35 mm film) are referred to as "35-mm-equivalent" cameras. The focal lengths of lenses mentioned in this book apply to 35-mm-equivalent cameras. Users should note that many DSLR camera bodies use sensors that are smaller than "full frame" sensors, thus the effective focal length of these lenses will be greater than on a 35-mm-equivalent body. The effective focal length of a 35-mm-equivalent lens can be calculated by multiplying the focal length of the lens by the "crop factor" of the image sensor. Many high-quality DSLRs have crop factors of about 1.5×, giving an 85 mm lens an effective focal length of ~130 mm.

The ideal lenses for photographing osteological specimens are "flat-field" lenses — those with focal lengths about twice that of "normal" (70–120 mm). The optical qualities of these lenses produce a flat field of focus without the characteristic linear distortions of wide-angle lenses or the depth compression of telephoto lenses. A 105 mm macro lens is ideal for general-purpose osteological photography.

Lenses with macro-focusing capabilities (also called "close-up" lenses) are useful in documenting small specimens. These lenses allow the focal point of a lens to extend farther from the film plane, effectively enlarging the subject. For example, macro lenses with a 1:1 capability can record a tooth life-size on the film, thus maximizing detail.

High-definition (HD) digital video is becoming common in the field as camcorders get smaller and more affordable. Video tape has been the common recording media, but solid-state "flash" cards are increasingly found on video cameras.

Camcorders with progressive scan capability also have a single-shot mode that can serve as an emergency back-up for stills. Capturing a single video frame for use in print or still-image presentation from progressive scan is easier and at a higher quality than is possible from interlaced video. Single frame captures from interlaced video benefit from the application of a special 'de-interlace' filter in Adobe Photoshop™.

Equipment maintenance is extremely important in the field. Environmental considerations are often neglected until it is too late. Heat, sand, cold, and moisture all wreak havoc on camera equipment. Fungal growth on internal lens elements is common in very humid environments. Dry and windy conditions require diligent protection from blowing sand and the harsh effects of heat on equipment and media. Extreme cold can defeat batteries quickly in electronic cameras. Lithium and other specialized batteries may be difficult to find away from cities. All of these concerns are exacerbated in digital photography. There is no mechanical backup solution in digital photography. For workers involved primarily in remote field work, a mechanical film camera is still necessary, at least for backup.

16.7.2 Exposure and Film (or other Recording Media)

The sensitivity of film and digital sensors to light is rated by an ISO number — a higher number means it is more light-sensitive. In film photography, one chooses a film with a particular ISO according to anticipated conditions. If conditions change, one is constrained by the limits of that film until the entire roll is exposed and developed. In digital photography there is no developing. The desired ISO for the conditions can be dialed in and adjusted at any time.

The interrelationship of ISO, lens aperture, and shutter speed matters in a correct exposure. Good photographers are aware of this three-item juggling act at all times, even with the sophisticated metering schemes in many cameras. The "sunny 16" rule serves as a practical starting point. It means that on a clear sunny day with a lens aperture of f/16, the shutter speed will equal the ISO rating of the film.

- **Lens aperture** controls how much of the subject closer to or farther from the camera is in focus (depth of field), as well as the *amount* of light that reaches the film. Small apertures create a greater depth of field, but less light gets through. Large apertures cause more of the foreground and background to be out of focus. Because the aperture (or f-stop) is expressed as a ratio of the diameter of the hole to the focal length of the lens, f/22 is smaller (less light) than f/4.
- **Shutter speed** controls how the motion of the subject is recorded and the *duration* of the exposure. A bone in the sand affords a slow shutter speed in order to achieve a small aperture for greater depth of field. However, hand-holding the camera at a slow shutter speed can cause camera shake and a blurred image. The rule is: hand-hold only with a shutter speed number larger than the focal length of the lens. This means 1/125 sec. with a 105 mm lens. Slower shutter speeds require the use of a tripod to insure a sharp image.

- **ISO** is all about the *sensitivity* of the media. Cloudy days and photographing in shadows require higher sensitivity. Low-ISO films contain finer grains of light-sensitive crystals that produce sharper images with higher contrast, but require longer exposures, wider apertures, or both. High-ISO films are faster, but can be grainy, flat in contrast, and preserve less detail. In digital photography, lower ISOs are optimal. Highest-ISO settings only amplify the signal from the sensor. This can produce digital "noise"—undesired random pixels scattered about an image. Most professional cameras have an ISO range up to 1600 or 3200, then offer a couple of higher settings referenced as H1 and H2. It is in these additional settings where the camera sensor's abilities are "pushed" and digital artifacts can be introduced into the image.
- The marked full steps of aperture, shutter speed, and ISO on most cameras and lenses double or halve the exposure with each step. From f/16 to f/11 doubles the light through the lens; from 1/125 sec. to 1/250 sec. the duration of the exposure is cut in half; and a film rated at ISO400 is twice as sensitive to light as ISO200.
- Use of a **gray card** (18% reflectance) is the best way to set a proper exposure in most conditions. Light meters are calibrated to this standard. You can meter from the camera filling the viewfinder with the gray card or you can point a hand-held light meter at an evenly-illuminated card (devoid of shadows or glare). Gray cards are available at any camera store catering to the professional and can be cut down to a convenient pocket size.

Digital photography offers huge advantages over film while traveling. Film—of any ISO, exposed or unexposed—should never be x-rayed despite what signs may say on the machines at security check points. Exposure to airport x-rays are cumulative and affect faster ISO films the most. Do not leave film in checked luggage as x-ray machines used to scan checked luggage often use higher doses of x-radiation. Always have film hand-checked at security. X-rays do not affect digital images or storage media, though strong magnetic fields may corrupt some digital media.

Lower-end digital cameras record images only in JPEG format, usually with an option of high-, medium-, or low-quality compression ratios. JPEG is a "lossy" algorithm that discards information as it compresses files. It can introduce artifacts into an image which are compounded with each compression/decompression. Though not meaningful in snapshots, these can have ramifications in critical science photography, particularly images used as forensic evidence. The universal workhorse TIFF format is available on many cameras, but produces larger files. Professional cameras can record in 12-bit "camera RAW" format that produces huge files. This uncompressed format contains all the information captured by the camera without in-camera image processing. It includes time, date, frame number, lens, and exposure information. It can be customized to include other information such as distance to the subject and GPS latitude/longitude data.

Pristine image data in RAW files provide the greatest flexibility. However, RAW files require processing down the line, including sharpening and adjusting contrast and color levels. It takes some practice to get the technique correct. Nikon, Canon, and other professional lines offer proprietary software designed to simplify processing RAW images. Similar results are obtainable using Adobe Photoshop and Apple's Aperture. Good cameras have the option of recording a RAW image and a processed JPEG image at the same time. This is the best of both worlds. It captures the maximum amount of image data while providing an immediately usable image.

A variety of capture media exist including Memory Sticks™, Microdrives®, and wireless connections to computer hard drives. CompactFlash (or CF) cards are proving to be robust and safe storage devices. CF card capacity keeps expanding each year. They range from 2 MB to 128 GB. As media technology advances, the high-capacity cards become increasingly faster at recording and downloading. This is important for large image files or a "burst" exposure sequence. A slow media card can pause shooting at critical moments as it accepts data from the camera's processor.

Images on memory devices in a camera are easily downloaded to a computer hard drive directly from the camera, typically using a USB cable, or via a separate multi-format card reader. It is important to archive digital images on CDs or DVDs at their maximum quality before editing.

Slides and negatives should be stored in polypropylene sleeves. Metal boxes for slides and glassine envelopes for negatives are acceptable. Avoid using vinyl slide sheets as the off-gassing of

the vinyl will destroy dyes on film. Rare and valuable images, both prints and slides, should be duplicated and/or scanned and then locked away under archival conditions. Repeated projections of slides and exposure of all film and prints to the elements degrades their quality over time.

16.7.3 Lighting and Setup

Proper orientation and lighting separate the professional-looking photograph from snapshots. Tripods and electronic flashes are fundamental for good photographs. Field photography primarily relies on available natural light, but the quality of light changes with weather and time of day. Photographs taken very early or late in the day often involve shadows that can be mitigated with an electronic flash. Fill flashes can reveal detail in deep mid-day shadows and reduce harsh image contrast. White cards, T-shirts, and crumpled aluminum foil can be used to bounce softer light into dark shadows. Manipulating light to come from the side of a subject will emphasize detail and texture. An off-camera flash sync cord is convenient for this purpose. A straight-on flash will "flatten" an image by eliminating most shadows. Ring flashes are used in forensic and medical photography for flat, even illumination.

A little thought about setting up shots will improve their value. Backgrounds should contrast with the subject. Spraying water lightly on the matrix around fossil bones *in situ* helps create contrast. In the lab, black or gray velvet or felt backgrounds improve color accuracy. Specimens can be stabilized and presented in a proper plane of reference with things as simple as a set of rubber washers. Avoid oil-based clays that contaminate specimens. A reference scale should appear with all specimens. Geological hammers, coins, and lens caps are poor substitutes for an easy-to-read metric scale. A scale is most useful when raised to the focal plane of the important part of the subject.

16.7.4 Legal and Ethical Considerations

Photography can be a powerful tool when an osteologist is called upon to serve as an expert witness (see Section 17.3). All photographic evidence presented in court is subject to cross-examination, including the manner in which it was acquired, archived, and reproduced. "Admissibility" of evidence is different from "weight" of the evidence. Some jurisdictions question the admissibility of digital photography due to the ease of manipulating the image, preferring film-based images instead. Others simply consider that all photography goes to the "weight" of the evidence.

If it is expected that your photography will be used to support opinions in a legal context, the best practice is to shoot film to establish a basic record and then take advantage of the flexibility and convenience of digital photography. A "throw-away camera" is often sufficient in this regard, constituting inexpensive insurance. As attorneys and courts become more aware that forensic image analysis can identify manipulated digital images, they are less likely to challenge digital pictures when they are initially captured in RAW format and backed up with conventional film.

Concerns have been raised in both academia and government about the manipulation of digital images and how it relates to data integrity. Image manipulation is not the same thing as image falsification—sharpening an image or making tonal adjustments are important parts of basic image processing. However, the ease of manipulating and recomposing images with image editing software like Photoshop has raised serious concerns among science ethicists and journal editors (Pearson, 2005). In 1989–1990, prior to the introduction of Photoshop, the U.S. Office of Research Integrity (ORI) reported that only 2.5% of investigated allegations involved images (Krueger, 2002). The percentage of image-involved cases steadily increased through the 1990s, reaching 14.3% in 1999–2000. The absolute and relative frequency of image manipulation cases continued to grow in the 2000s, hitting 30.3% in 2001–2002, 44.1% in 2005–2006, and reaching 68% in 2007–2008 (Krueger, 2008, 2009; Gilbert, 2009).

The editors of *The Journal of Cell Biology* examined the images in every paper submitted from 2002 to 2006 (NAS, 2009). They found that over 25% of all received manuscripts contained at

least one inappropriately manipulated image, although 95% of these were determined to be innocent of fraudulent intent.

The growing epidemic of inappropriate image manipulation has led many journal editors to develop image manipulation guidelines (*e.g.,* Scott-Lichter et al., 2009). The editors at Rockefeller University Press (Rossner and Yamada, 2004) established four basic guidelines which have subsequently been adopted by many other journals (Scott-Lichter et al., 2009):

1. No specific feature within an image may be enhanced, obscured, moved, removed, or introduced.
2. Adjustments of brightness, contrast, or color balance are acceptable if they are applied to the whole image and as long as they do not obscure, eliminate, or misrepresent any information present in the original.
3. The grouping of images [from different parts of the image or from different images] must be made explicit by the arrangement of the figure (*e.g.,* dividing lines) and in the text of the figure legend.
4. If the original data cannot be produced by an author when asked to provide it, acceptance of the manuscript may be revoked.

In addition, several major journals have adopted a fully digital workflow in order to be able to examine all submitted images for signs of improper manipulation (Gilbert, 2009; NAS, 2009). For readers of journals that have not yet implemented image verification procedures, ORI offers free forensic tools for detecting manipulated images at *http://www.ori.dhhs.gov/tools/*. Due to the nature and scope of the problem of image manipulation, Krueger (2008) notes that manuscript review now essentially "extends to all, includes the public, and lasts indefinitely!"

Archiving unaltered RAW digital images (or even taking a few film shots) is a necessary measure should you ever need to defend the integrity of images used to tell an important story. Suffice it to say, immense caution is warranted when one chooses to modify an image to enhance a scientific point. See Gilbert and Richards (2000) for a discussion of digital imaging of bone modification and the ethical question of digital alteration.

16.8 Radiography

Analysis of bones in the living individual is usually accomplished by exposing a film to x-rays passed through the body part. The bone tissue blocks some x-rays, resulting in a negative image on the film called a **radiograph**. Because bones, including internal parts of bones, block some of the rays, the radiograph can be a valuable aid in diagnosing bone conditions in medicine.

Standard radiography is also a valuable tool for the osteologist. Because there is no risk to a dry bone specimen, various exposures and orientations may be made to show the internal architecture of a bone or the developmental status of an unerupted dentition. Osteologists often use fully enclosed x-ray devices within shielded, benchtop cases with external controls (Faxitron® or other). The specimen should be oriented so that the x-ray beam passes through the center of the area of interest and so that this area is perpendicular to the beam and parallel with the film plane. The specimen and film should be as far as practical from the x-ray source and as close to one another as possible. Computer-assisted enhancement of radiographs may be useful after processing (Odwak and Schulting, 1996). The development of computed tomography (CT) scanning adds a potent bone-sectioning tool to the osteologist's kit. Medical (or clinical) CT scanners are usually only found in dedicated rooms in hospital radiography departments, and their use requires collaboration with personnel therein.

Radiographic technologies originally developed for industrial purposes have even better resolution and can be used in a variety of settings. As these technologies and methodologies mature, the potential applications of radiographic analyses are increasing. The ability to take accurate measurements from radiographic images or their derivatives is one example. With industrial CT

scanning (also called microfocal CT or micro-CT scanning), accuracies of less than one tenth of a millimeter are routine. Spoor et al. (1993) discuss applications and problems involved in the derivation of osteometric data from CT scans. Olejniczak and Grine (2006) examine enamel thickness measurements from micro-CT scans and find them accurate except when the enamel is less than 0.1 mm thick and/or in heavily fossilized teeth. Suwa and Kono (2005) caution that buccolingual locations of enamel thickness measurements must be carefully selected, and molar position carefully controlled, to ensure accurate results. Olejniczak et al. (2007) verify that dental measurements taken with different types of micro-CT scanners are comparable unless the teeth are heavily fossilized.

Rühli et al. (2007) compare the utility of micro-CT scanning to traditional histological sectioning for pathological diagnosis. They conclude that micro-CT has several advantages over sectioning, but because of the difficulty inherent in differentiating woven from lamellar bone with micro-CT scans, both techniques should be used for best results. Robinson et al. (2008) explore the forensic and research potential of taking osteological measurements from 3-D reconstructions of undefleshed remains, and find the accuracy comparable to traditional measurements of the same bones, once defleshed. Recently, even more powerful synchrotron-based scanning has provided microscopic resolution on osteological and dental remains. The biological effects of such scanning on tissues with residual organic molecules have only recently begun to be assessed, but it is already clear that the more ionizing radiation a specimen receives, the more damage it will incur. The fact that damage assessment is coming only after so many rare specimens have already been subjected to high radiation loads is of considerable concern in an era when more and more can be learned from the molecules at risk from such procedures.

When possible it is always better to make observations and take measurements directly from the specimen. This is because measuring from a radiograph or CT scan greatly increases the chance that matrix, clothing, distortion, or erosion will be overlooked and inaccurate measurements generated. To use any of these techniques in investigating human skeletal material, consult a specialist in radiography. See Ortner (2003), Hillson (1996), and Bruwelheide (2001) for discussions of radiography in osteological and dental analyses, and Mafart and Delingette (2002) and Mafart et al. (2004) for applications of three-dimensional imaging in paleoanthropology and prehistoric archaeology.

16.9 Microscopy

Fine details on the surface of a bone or tooth may be best investigated with a binocular dissecting microscope. Intense, unidirectional light sources can be used to emphasize microscopic detail. For discriminating between various kinds of surface alteration on bones (root marks, cut marks, pathology), the binocular microscope is a most valuable aid. To photograph microscopic structure or trauma on the surface of a bone, the scanning electron microscope (SEM) can provide excellent images with great depth of field. For large specimens that do not fit in the vacuum chamber of the microscope, or for specimens housed in institutions without SEM facilities, it may be necessary to replicate the surface of the object by molding it with dental impression rubbers and pouring an epoxy positive for use in the analysis. For work on bone or tooth surfaces at high magnification, the SEM has been the traditional tool of choice (and, unfortunately, expense), but alternative digital imaging technologies may change this (Ungar et al., 2003; Gilbert and Richards, 2000).

Standard histological microscopic techniques, micro-CT, or synchrotron scans are used to study the microscopic structure of bone below the surface or the internal structure of teeth (Hillson, 1996). Schultz (1997a, b) provides two reviews of how microscopy can be applied in human osteological studies. Mahoney (in press) illustrates the richness of growth data available through a microscopic examination of deciduous molar enamel. Histological age determination using light microscopy is becoming a reliable tool in the osteologist's toolkit (Section 18.3.11). The diagnostic value of microscopy in paleopathology is well-established (see Chapter 19).

16.10 Molding and Casting

Casts of skeletal material are used in osteology and paleontology for several purposes. They provide a good three-dimensional archival record of the object under study (Mann and Monge, 1987; Smith and Latimer, 1989). In addition, they are useful in communicating findings with colleagues and for slicing into cross sections for comparative purposes. Specialized techniques and materials used in molding and casting teeth are outlined in Hillson (1992, 1996). Several molding methods may be used for bones, depending on the needs of the investigator. Some of the more common molding methods and materials are listed here:

- **Alginate impression material.** Powders mixed with water form material that dentists use to make impressions of teeth. Alginates are good for making quick, one-sided molds and are widely available, inexpensive, and easy to use. They are not recommended for holding very fine detail or for the production of more than one or two casts. The molds deteriorate within days even when kept moist.
- **Latex rubber molds.** This material gives higher resolution and the ability to make two-part molds. The material is more expensive than dental impression compounds but lasts much longer.
- **Silicone molds.** Silicone elastomers come in three basic varieties: alcohol-based evaporative cure silicone, tin-catalyzed condensation cure silicone, and platinum-catalyzed addition cure silicone. Platinum-catalyzed silicones like Dow Corning's Silastic are the material of choice for mold detail and longevity. This material, however, is the most costly and can be time-consuming to use.
- **Polyvinylsiloxane impression materials.** Quick-setting, auto-mixing, injectable, flowable impression materials widely used in dentistry and in making void-free, high-fidelity, long-lasting molds. Commonly used for making molds for epoxy casts to be used in the scanning chamber of scanning electron microscopes.
- **Dental putties.** Two-part putties that are kneaded together by hand (parts are usually differently colored to aid in mixing) and used to make quick-setting impressions of small portions of bone surfaces.

Once removed from the original specimen, the resulting mold represents a three-dimensional "negative" of the original. If a self-hardening material is introduced to the mold and allowed to set or cure, the flexible mold can be carefully peeled away from the hardened cast to reveal a three-dimensional replica (or cast) of the original specimen. With a little care, the mold can be reused to make numerous additional casts.

As with molds, many kinds of materials can be used to make casts. The easiest casting material to use is plaster, which comes in many varieties. Some of the most common casting materials include the following:

- **Plaster of Paris.** Common Plaster of Paris is ubiquitous and inexpensive but it is soft and easily scratched, and it does not capture detail well.
- **Dental stones.** High-strength, fine-grained gypsum cements (dental stones) are harder, especially when mixed with hardeners instead of water. The resulting casts are quite hard, but also brittle, and can shatter if dropped. Detail on casts in these materials can be excellent, shrinkage is minimal, and the material is inherently stable once set.
- **Polyurethane plastics.** Polyurethane plastics are made by mixing two monomers together, usually in equal volumes. The monomers react by cross-linking, a process which ends in a hard plastic cast. Polyurethanes are not as stable as dental stones, and can degrade when exposed to direct sunlight or to organic solvents.
- **Epoxies.** Epoxies are thermosetting plastics that are activated by combining a resin and a hardener. Depending on the formulation, they can set quickly (generating a lot of heat)

or very slowly (producing minimal heat). Epoxies are commonly used for making casts of teeth for use in scanning electron microscopy.

There are various techniques for coloring casts of all materials to bring out detail. Most involve a vehicle such as alcohol for dissolving and spreading artists' pigments. Artists' fixatives and spray lacquer add a long-lasting, appealing, and protective finish to the final product.

Both computerized tomographic (CT) scanning and 3-D laser scanning can be combined with stereolithographic output to allow osteologists to perform "digital molding and casting" of hard tissue specimens, with the added benefit of revealing internal structure (Mafart et al., 2004; Mafart and Delingette, 2002).

16.11 Computing

The computer revolution continues to impact all areas of science, including human osteology. Satellites communicate with hand-held GPS (Global Positioning System) receivers to determine precise location (increasingly expressed in UTM or MGRS coordinates or in conventional latitude and longitude as decimal degrees or degrees and decimal minutes). Ruggedized laptop computers connected to electronic distance-measuring devices allow laser-precision in plotting specimens in the field. Desktop computers receive, process, and output our thoughts, our data, and our images. 3-D laser scanners connected to computers allow the external form of objects to be imaged in three dimensions, imported to the computer, and manipulated digitally in many ways (Figure 16.6). Computerized medical imaging allows us to peer deep within osseous structures to see formerly hidden evidence of ancient pathology. The exploding global communications network makes it possible to exchange ideas and data rapidly across international frontiers and between field and laboratory. As a result of all of these developments, it is impossible to think of working in human osteology without the aid and working knowledge of computer technologies.

In addition to basic word-processing skills and the programs necessary for scholarly communication, all osteologists should learn the basics of database and spreadsheet programs that allow for the rapid and easy manipulation of large osteological data sets. Unfortunately, human osteologists have not been immune to the false hope that computers and technology can replace real specimens and real expertise. The current high-tech craze has created some interesting exercises. For example, bones have been imaged by CT and laser scans, input to desktop and mainframe systems, manipulated therein (with attendant beautiful colors and bones floating and rotating in space), and then copied by sculpting the digitized bony form into plastic with stereolithography. Some such exercises are useful to surgeons customizing prosthetic devices and to investigators assessing fragile remains in matrix (Lynnerup et al., 1997). However, some of this high-tech wizardry applied to archaeological and paleontological specimens leaves the observer to conclude, "That was really awesome, but so what?" Computers can do incredible things, but they are tools that help us investigate, organize, and document. They do not substitute for our own imagination or critical judgment when assessing osteological remains.

16.12 Reporting

After the usually unpublished initial field reports are submitted to granting agencies and various governmental regulatory agencies, published reporting of hominid osteological remains from paleontological contexts often occurs in three stages: first, announcement in a prominent international journal such as *Science* or *Nature*, followed by anatomical description in a more specialized journal such as the *American Journal of Physical Anthropology*, and finally, usually years later, full monographic treatment. Basic metric, preservational, and contextual data are reported, along with interpretations, in all three publication venues.

In forensic human osteology, the reporting of skeletal remains usually follows a different series of steps. Here, because of the rigorous procedures adopted by law enforcement agencies and medical examiners (Komar and Buikstra, 2008), the osteologist's report becomes part of the legal record instead of moving straight toward publication. Whereas brevity and conciseness are required for the publication of osteological reports, clarity and thoroughness are of primary importance for the final reports of forensic anthropologists.

There is not a single format for forensic reporting in the United States, but such reports typically follow the standards set forth in Buikstra and Ubelaker (1994), are written in a narrative style, and usually follow the format of the JPAC Central Identification Laboratory (JPAC-CIL, 2008). In the analysis of a single set of remains, a forensic anthropologist may generate 10–20 pages or more of inventorial, observational, osteometric, and diagnostic data, usually on forms (data sheets) similar to those reproduced in the Appendix of Buikstra and Ubelaker (1994). These data sheets, along with a 3–5 page narrative summary, comprise the final report of the forensic anthropologist. The cases included in Steadman (2009) provide a good overview of the range of cases in which a forensic osteologist might be asked to participate.

In a forensic setting, the pressure for accurate and immediate reporting is sometimes very intense. Osteologists may be forced to conduct their examinations in suboptimal conditions—at morgues, in criminal laboratories, and even in refrigerated trucks or warehouses at the disaster scene. Pressure may come from the sensitivity of the case, from relatives wishing to conduct funerary rites, or from law enforcement agents requiring quick answers to pursue their investigations or hold their suspects. Under these conditions of inadequate facilities (including inadequate comparative materials) and intense pressure, osteologists are more prone to make mistakes. Suffice it to say that there is no tolerance for such mistakes in a forensic context, whatever the conditions. The osteologist should always state only what is defensible in a court of law, keep speculation to a minimum, and work closely with others on the multidisciplinary investigation team.

In archaeological osteology, the collaborating archaeologist and osteologist usually work out a reporting procedure in advance of the excavations and determine what information should be made available in reports or publications. The publication of the volume *Standards for Data Collection from Human Skeletal Remains* is a milestone in the standardization of data collection for osteological remains from archaeological contexts. This 1994 volume, realized only under the pressure of federal legislation forcing imminent destruction of osteological collections (see Chapter 17), contains a series of chapters and appendices (inventory forms for adult and immature remains) that provide a framework for the observation and recording of osteological attributes. It is an invaluable resource for the osteologist practicing in an archaeological context.

The following points are offered as a general guide to reporting on human osteological material. Most osteological reports, particularly in forensic settings, cover the points outlined here:

- **Introduction**. The osteologist should note when and how first contact was made regarding the case. The nature of the materials received or observed should be noted here. Any steps taken by the osteologist to preserve or otherwise alter the material should be outlined.
- **Bones present.** This is simply a listing of what bony remains were analyzed, sometimes with MNI determinations and their explanations included.
- **Context and condition of the remains.** This is particularly important in forensic and archaeological work. Note should be made of the context in which the bones were found. Remember that all of the remains received for analysis constitute evidence, often crucial and always irreplaceable. In particular, any cultural or biological remains associated with the bones should be noted. Soft tissue adhering to the bones should be described. Before removal of any soft tissue remains, check with a forensic pathologist about sampling of this material. Any soft tissue present should be radiographed extensively before removal to check for objects within (bullets, clothing, etc.). Never dispose of any associated material without consulting the officials involved in the investigation.

- **Pathology.** Assessment should be limited to the hard tissue. Note any evidence of bony pathology and leave the soft tissue to other experts. Note healed fractures and other osteological manifestations of disease.
- **Anomalies.** Report anything unusual about the skeletal remains, such as supernumerary digits or other nonmetric traits. These facts may help in individuation. In assessing radiographs, note any features that might be compared to antemortem films and thereby establish identity.
- **Trauma.** Report any signs of osteological trauma, ranging from healed fractures to excavation-related fractures. Try to determine how recent the fractures are by noting evidence of healing, color differences, or root-mark etching on broken surfaces. Express an opinion on whether the bone was fresh when broken (perimortem fracture) or dry (postmortem or 'nonvital'). Distinguish between pre- and post-depositional trauma when possible (Maples, 1986).
- **Age, sex, race, stature, and weight.** For these, be as specific as possible, but do not give estimates whose precision is not warranted. Give the appropriate limits of confidence in all determinations. Tell what methods were used to make the estimates and why these methods were used.
- **Time and cause of death.** Osteologists are almost never able to make these estimates with certainty. Whereas experienced investigators may speculate on time of death by using odor, grease, tissue, or bone weathering, these attributes all vary according to temperature, humidity, and cover. And how can the osteologist examining a gunshot through the head know that the victim was poisoned before being shot? For these reasons, the osteologist must work closely with a professional forensic pathologist and strictly avoid speculations about death based on bony evidence in isolation. By studying healed lesions the osteologist can sometimes say whether a person survived a skeletal trauma, but unhealed lesions often do not, by themselves, indicate the cause of death. The osteologist's legal contribution is usually limited to identification, sometimes including individuation. The skeleton itself gives little evidence relevant to questions about the time and cause of death.
- **Personal Identification**. This is the determination of the personal identity of the remains. The best hope for individuation, without soft tissue indicators such as fingerprints, is in dental records. For skeletal material lacking dental evidence for individuation, it is often possible to match pathological lesions or antemortem photographs or radiographs with postmortem images of the bone. Positive identifications may be based on old fractures or discrete trabecular or sinus patterns (Webster et al., 1986). See Wilkinson (2004) and Reichs and Craig (1998) for discussions of techniques used in facial "reproduction" (approximation) from a dry skull. Techniques for digital facial reconstruction are explored in Clement and Marks (2005). Individuation is often important in legal and insurance matters.
- **Metrics and nonmetrics.** Report standard dental, cranial, and postcranial measurements, as well as observations of nonmetric traits.
- **Summary.** Simply summarize the most significant conclusions reached for the sections given here.

Osteological findings of general interest are usually reported in a scientific publication that makes data available to the scientific community as a permanent record. In describing the results of osteological analysis, communication must be unambiguous. The osteologist should specify exactly what materials were analyzed, what procedures were used in the analysis, and what results were achieved. Most scientific publications have basic sections of "Introduction," "Materials and Methods," "Results," "Conclusions," and "Bibliography." Scientific papers on human osteology are found frequently in book or monograph form as well as in journals such as the *American Journal of Physical Anthropology,* the *International Journal of Osteoarchaeology, Forensic Science International,* and the *Journal of Forensic Sciences.*

16.13 Curation

During the initial processing of skeletal material, it is advisable to label all bones individually with a prefix designating the site and a number representing the skeletal individual. For example, ARA-VP-1/1 is a specimen number for the first vertebrate paleontological specimen from the first locality collected in the Aramis area of Ethiopia in 1992. It is crucial that this labeling be legible, with numerals that anyone can read. Be very careful not to confuse 9s with 2s or 1s with 7s. Specimen numbers should be written in permanent, waterproof ink and protected with a layer of B72 once the ink is completely dry. For softer bones, it may be necessary to let a drop of consolidant dry and harden on the bone surface before putting the label on the bone. This treatment prevents the ink from diffusing into an illegible blob. Specimen numbers are essential; they represent the critical links between bones and information on their original context. Mixing of labeled material is very bad practice in the laboratory, but mixing of unlabeled material is often irreversible, and therefore unforgivable. Labels should be put on bones early in the curatorial process.

There are two main objectives of curation. The first, as in other steps outlined earlier, is to prevent loss of information. Information loss can come in the form of actual physical destruction of the bones and teeth, in the mixing of unlabeled elements in the collection, or in the loss or destruction of the records (paper or digital) for the skeletal material. Almost all simple breakage of bones can be repaired with glue. The objective of curation, however, is to prevent breakage in the first place by handling and storing bony material properly. Untrained or unqualified persons should not handle osteological material without supervision. Metric or photographic analysis of material should not be allowed to damage the specimens. Bones are fairly tolerant of a range of storage conditions, but their containers (boxes, trays, bags, padding) should be composed of a nondeteriorating, acid-free material. Bones should be stored in areas in which humidity, incident light, and extreme temperatures are kept to a minimum. Steps should be taken to see that insects and rodents are kept away from stored skeletal material and records. To prevent accidental loss of records from flooding, fire, or theft, it is advisable to make a copy of all skeletal records (either digitally or as a hard copy) and to store this copy in a separate location.

The second major role of curation is the provision of research access to the collection. To provide this access, it is necessary to impose and maintain a high degree of organization in the skeletal collection. A researcher should be able to move quickly and efficiently between the bony remains and their records. Computer databases are important, not only for collection organization, but also as a means of enhancing research access. Cassman et al. (2007) and Caffell et al. (2001) provide overviews of curatorial procedures and problems in human osteology.

Suggested Further Readings

Adams, B. J., and Byrd, J. E. (Eds.) (2008) *Recovery, analysis, and identification of commingled human remains.* Totowa, NJ: Humana Press. 374 pp.

This edited volume covers techniques used in MNI determinations and forensic individuation. Methodologies covered include contextual analysis, sorting, statistics, GIS, radiology, and DNA analysis. The volume focuses on forensic contexts but includes several archaeological case studies.

Buikstra, J. E., and Ubelaker, D. H. (Eds.) (1994) *Standards for data collection from human skeletal remains.* Fayetteville, AR: Arkansas Archaeological Survey Report No. 44. 206 pp.

The essential osteological standards volume in North America.

Chhem, R. K., and Brothwell, D. R. (2008) *Paleoradiology: Imaging mummies and fossils.* New York, NY: Springer. 163 pp.

Six chapters cover the current developments and history in the field of radiology and CT imaging of both paleohuman and nonhuman subjects. Includes advice on protocols, techniques, expected error, and interpretation of radiographic data.

Hillson, S. (1996) *Dental anthropology.* Cambridge, UK: Cambridge University Press. 373 pp.

Appendix A provides a good guide to field and laboratory methods used to extract, dissect, replicate, image, section, and preserve dental remains.

Katzenberg, M. A., and Saunders, S. R. (Eds.) (2008) *Biological anthropology of the human skeleton* (2nd ed.). Hoboken, NJ: John Wiley and Sons. 680 pp.

Introduces numerous osteological subspecialties, illustrates their methods and presents case studies for each. Covers the history, new directions, and tools for each field.

Mead, E. M., and Meeks, S. (1989) Photography of archaeological and paleontological bone specimens. In: R. Bonnichsen and M. H. Sorg (Eds.) *Bone modification.* Pp. 267–281. Orono, ME: Center for the Study of the First Americans.

This paper has a special orientation to the photography of bones and is therefore of value to the osteologist.

Morton, R. A. (Ed.) (1984) *Photography for the scientist* (2nd ed.). London, UK: Academic Press. 542 pp.

A complete guide to the subject, with many advanced techniques.

Reichs, K. J. (Ed.) (1998) *Forensic osteology: Advances in the identification of human remains* (2nd ed.). Springfield, IL: C. C. Thomas. 567 pp.

Trauma analysis is covered in Section VI, and Section V covers aspects of building a biological profile. There are also chapters on facial approximation and the use of statistics in forensic anthropology.

Schwartz, J. H. (2006) *Skeleton keys: An introduction to human skeletal morphology, development, and analysis* (2nd ed.). New York, NY: Oxford University Press. 416 pp.

General textbook for osteology. Includes a CD-ROM with photographs of skeletal elements, growth series, and examples of peri- and post-mortem trauma.

Slice, D. E. (Ed.) (2005) *Modern morphometrics in physical anthropology.* New York, NY: Kluwer Academic/Plenum Publishers. 384 pp.

An introduction to anthropological morphometrics and its potential applications, as well as a review of 20 years of methodological improvements.

Smith, J., and Latimer, B. (1989) A method for making three-dimensional reproductions of bones and fossils. *Kirtlandia: Journal of the Cleveland Museum of Natural History* 44:3–16.

A good introduction to molding and casting techniques used with modern and fossil osteological material.

Steadman, D. W. (Ed.) (2009) *Hard evidence: Case studies in forensic anthropology* (2nd ed.). Upper Saddle River, NJ: Prentice Hall. 360 pp.

An engaging and well-illustrated collection of 25 case studies, presenting a wide range of forensic situations addressed with current and often innovative approaches.

Urdan, T. C. (2010) *Statistics in plain English* (3rd ed.). New York, NY: Routledge. 223 pp.

An approachable introduction to statistical principles and methodology.